Effects of Clinical and Subclinical Hyperthyroidism on Some Lipid Profiles Among Sudanese Females

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(وَنِنزَلَ مِنَ الْقُرْآنِ مَا هُوَ شَفَاءٌ وَرَحْمَةُ

الآية 82 - الإسراء)
Dedication

To my parents, who mean everything to me.

To my lovely kids; Braa, Abdelrahman and sahar

To my husband for the endless support

To my brothers and sisters

With my love.
Acknowledgments

First of all I would like to thank our god; Allah who creates us from nothing. The efforts of my supervisor Dr. Nabiela Musa El Bagir should be appreciated. She has been so patient throughout this research with good ideas and great comments and recommendations. Thanks are also extend to the staff of RIA labs in Sudan Atomic Energy Commission (SAEC) and Radio Isotopes Centre, Khartoum (RICK) for their great help in samples collection and providing us with their records for the period 1994 to 1996 for the survey. Thanks to my family for their continuous support and encouragement to do this research which I hope to be of some value. Also, my thanks should go to those who helped me throughout my life. Lastly but not leastly, my thanks should go to Ammar Mohammed Elamin Hassan of RIA lab, SAEC for his continuous support and help to finish this research.
Abstract

The first part of this work was a survey conducted on the patients referred to the Radio immuno assay (RIA) laboratory of Sudan Atomic Energy agency (SAEC) 1994-1996. The total number of the referred patients during the three years was found to be 4268 subjects, 85% of them were referred for the first time. The survey revealed that 87% of the patients referred to the lab. were females and 12.6 only were males. The incidence of thyroid disorders were found to be also higher in females which comprised (33%) of the study group compared to (29%) males. The analysis of blood lipids which was conducted in 120 females, to compare clinical hyperthyroid patients to subclinical cases, showed significant variations between the hyperthyroid group and the euthyroid one. This was presented as significantly lower levels of the total cholesterol and the lipoprotein cholesterols, LDL-C and HDL-C, in hyperthyroid patients, compared to euthyroid group, with significantly higher levels of triacylglycerols in the euthyroid group. The subclinical cases showed very low levels of the LDL-C and significantly higher levels of the triacylglycerols with similar value of the total cholesterol and the HDL-C compared to the euthyroid group. Results indicated that lipid metabolism in subclinical hyperthyroid cases was slightly affected by the hormonal disorder.
الخلاصة

Joshua Al-Audy and Ozen 2021
لا يمكنني قراءة النص العربي بشكل طبيعي. يرجى تقديم النص باللغة العربية.
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Introduction

Thyroid hormones have a substantial role in regulating different pathways of metabolism hence they are called metabolic hormones. The function of thyroid gland in turn is under the control of the pituitary gland through the thyroid stimulating hormone (TSH). The thyroid hormones maintain the level of metabolism in the tissues that is optimum for their normal function; they are well known to stimulate the oxygen consumption of most of the cells in the body, help to regulate lipid and carbohydrates metabolism, modulation of gonadotropin secretion by the pituitary and maintenance of proliferative cell growth and maturation in hair. In addition, thyroid hormones stimulate both sodium pump and glycolytic pathway leading to calorigenesis and oxidative phosphorylation in tissues such as liver, kidney, and muscle (Ganong, 1997).

Physiological concentrations of thyroid hormones exert both anabolic and catabolic effects promoting the normal metabolic turn-over, but higher concentrations exert catabolic responses. Disorders of the thyroid gland lead to disturbances in the different pathways that are regulated by its secretion (Murray et al, 2000). More secretion of thyroid hormones (hyperthyroidism) leads to body wasting, nervousness, tachycardia, tremor, muscle weakness (thyrotoxic myopathy) and excess heat production (Ganong, 1997). Subclinical thyroid dysfunction is a common clinical problem for which there are many controversial issues regarding screening, evaluation, and management. Subclinical hyperthyroidism and hypothyroidism have subtle clinical manifestations at most, and the importance of timely diagnosis and treatment continue to be contentious subjects of research studies, position papers, and editorials. Both Subclinical hyperthyroidism and subclinical
hypothyroidism have many clinical implications that can be seriously harmful if not treated. These include: progression to overt hypo and hyperthyroidism, cardiovascular diseases, atria fibrillation, osteoporosis and bone fracture (Helfand, 2004).

According to Tunbridge et al. (1977) and Hak et al. (2000), subclinical disorders are found to be common and twice as often in women as in men, this is one of reasons of targeting females in this study (Tunbridge et al., 1977 and Hak et al., 2000). Also females are more likely to have clinical thyroid diseases (Murray et al, 2000) and this is the second reason for selecting them for this study. It is well known that women have more lipids compared to men (Murray et al, 2000) and this is the third and last reason for choosing them for this research as we expect good image about how lipid metabolism is affected by disorders of the thyroid gland. However, in this research, women who are suffering from either clinical or subclinical hyperthyroidism; are going to be...
2. To estimate the levels of lipid profiles from blood samples of Sudanese females that are either suffering from clinical or subclinical hyperthyroid function compared to euthyroid Sudanese females.

3. To detect the effect of clinical and subclinical thyroid dysfunction on the lipid metabolism.
CHAPTER ONE

Literature review

1. The Thyroid gland

The human thyroid gland originates from the 4th and 5th branchial pouches, which fuse into a single structure and this tissue is present in all vertebrates and lies in humans at the root of the neck in front of the upper rings of the trachea. The gland has two lobes, right and left which are connected by an isthmus. Normal thyroid weight about 20 to 30 grams. Histologically, the gland consists of a multitude of follicles which are surrounded by connective tissue and filled with colloid. When the gland is active the follicles are small and the cells are cuboids while in inactive gland...
1.1.1 Iodine metabolism

Thyroid hormones are unique in that they require the trace element iodine for biologic activity. A complex mechanism has evolved to acquire and retain this crucial element and to convert it into a form suitable for incorporation into organic compound. The thyroid is able to concentrate I\(^\text{-}\) against a strong electrochemical gradient. This active transport system allows the human thyroid gland to maintain a concentration of free iodide 30-40 times that in plasma (Williams, 2001). The transport mechanism is frequently called the "iodide-trapping mechanism" or "iodide pump". The pump is an example of secondary active transport (Ganong, 1997). A very small amount of iodide enters the thyroid by diffusion. Also any intracellular I\(^\text{-}\) that is not incorporated into monoiodotyrosine (MIT) or diiodotyrosine (DIT) is free to leave by this mechanism (Murray et al, 2000).

Two thirds of the absorbed iodine is excreted in the urine within 2 to 3 days after ingestion, and may also be lost in the faeces, sweat and milk. Most of this iodine comes from the endant process and is linked to the ATPase-dependent Na+/K+ pump which in turn corrected primarily by TSH (Murray et al, 2000). I\(^\text{-}\) is transported across the basement membrane of the thyroid cell by an intrinsic membrane molecule called the Na+/I\(^\text{-}\) symporter (NIS). The NIS derives its energy from Na+/K+ ATPase, which derives the tans breakdown of thyroid hormones (Williams, 2001). The recommendations of the World Health Organization (WHO) for optimal daily iodine intake are as follows: 150 µg for adults, 200 µg during pregnancy and lactation, and 50 µg for the first year of life and increases as the age increases up to the adult dose (Greenspan and Gardner, 2004).

1.1.2 Thyroglobulin

μg of T₄/dl. TBG binds about 10% of the circulating T₄. Its affinity for T₃ is about 10 fold lower than for T₄, so that it mostly carries T₄. Albumin has one strong binding site for T₄ and T₃ and several weaker ones. Because of its high concentration in serum, albumin carries about 15% of circulating T₄ and T₃ (Williams, 2001).

1.1.5 Metabolism of thyroid hormones

The daily secretion of the normal thyroid gland is about 100 nmole of T₄, about 5 nmole of T₃, and less than 5 nmole of metabolically inactive reverseT₃ (rT₃) (Williams, 2001). T₄ and T₃ are deiodinated mainly in the liver, the kidney, and many other tissues. Two different enzymes are involved, 5-deiodinase catalyzing the formation of T₃, which is three to eight times more potent than T₄, and 5-diodinase catalyzing the formation of r-T₃, which is metabolically inert. T₃ and r-T₃ are then converted to various diiodothyronines (Ganong, 1997). Monodeiodination of the outer ring of thyroxine is a "step up" process; increasing the metabolic activity of the resultant compound, while monodeiodination of the inner ring is a "step down" or inactivation process. Most peripheral target tissues utilize T₃ that is derived from the circulating hormone. Notable exceptions are the brain and pituitary, for which local generation of T₃ is a major source for the intracellular hormone (Hardman and Limbird, 2001).

1.2 Physiologic effects of thyroid hormones

Thyroid hormones, considered as metabolic hormones that affect all organs development and physiology. 18ed. Edinburgh, London living stone.

References


5- Caparevic, Z.; Stojanovic, D.; Llic, V.; Bojkovic, G. and Stojanovic, M.
Conclusions

Form this project we come to a conclusion that lipid profile is affected by the status of the thyroid gland; where in hyperthyroid females the levels of all the fractions were shown to be significantly decreased due to the overactivity of the thyroid gland while in subhyperthyroid glands only triglycerides was found to be significantly decreased and the others were not affected and this can be explained by the fact that subclinical hyperthyroid affects the level of TSH but not the T4 and T3; and the later is the active hormones in metabolism.

Recommendations

1- To the RIA, of SAEC; to modify the registration form for the referred patients to include tribe, original residence, family history of the disease, economical status.

2- To make sure that age, sex and treatment are fully addressed.

3- To make use of samples to conduct research in the biochemistry and physiology of human being after getting the permission.

4- To do lipid profile testing for patients who have clinical or subclinical thyroid functions.

Haematopoitic effects: The increased cellular demand for oxygen in hyperthyroidism leads to increased production of erythropoietin. However, blood volume is usually not increased because of hemodilution and increased red cells turnover (Greenspan and Gardner, 2004).

Gastrointestinal effects: They stimulate gut motility. This may also contribute to the modest weight loss in hyperthyroidism (Greenspan and Gardner, 2004).

Skeletal effects: They stimulate increased bone turnover, increased bone resorption thus chronic hyperthyroidism may result in significant osteopenia and in severe cases modest hypercalcemia and hypercalciuria (Ganong, 1997).

Neuromuscular effects: Thyroid hormones increase the number and affinity of

65
minutes in a hyperthyroid patients and about 150 minutes in hypothyroid patients (Greenspan and Gardner; 2004).

1.3 Hyperthyroidism; thyrotoxicosis

Over-activity of thyroid gland is a clinical syndrome produced by sustained high plasma concentrations of thyroid hormones, may be easy to diagnose clinically or may remain unsuspected for a long time. It affects 2-5% of all females at sometime and with a sex ratio of 5:1 most often between ages 20-40 years. Nearly all cases (99%) are caused by intrinsic thyroid disease; a pituitary cause is extremely rare (Kumar and Klark, 2002, Longmore et al, 2001).

1.3.1 Causes of hyperthyroidism

- **Graves' disease.** The cause of most hyperthyroidism is Graves' disease, an autoimmune disorder in which antibodies produced by immune system stimulate thyroid to produce too much thyroxine.
  
  Normally, immune system uses antibodies to help protect against viruses, bacteria and other foreign substances that invade body. In Graves' abnormalities. Worthy to be noted, most of researchers and authors focused disease, antibodies mistakenly attack thyroid gland and occasionally the on other implications that related to subclinical hyperthyroidism. These tissue behind the eyes and the skin of lower legs over the shins. Scientists include atrial fibrillation, osteoporosis, and cardiovascular diseases.
  
  As mentioned before, the literature on assessment and treatment of patients with subclinical hyperthyroidism is markedly less extensive. These aren't exactly what causes Graves' disease, although several factors - including a genetic predisposition - are likely involved.

- **Hyperfunctioning thyroid nodules (toxic adenoma, toxic multinodular goiter, Plummer's disease).** This form of hyperthyroidism occurs when one or more adenomas of thyroid produce too much thyroxine. An adenoma is a part of the gland that has walled itself off from the rest of the gland, forming noncancerous (benign) Untied States population and the development of assays with enhanced TSH sensitivity.
Cholesterol level (172.7±31). In case of subclinical hyperthyroid female Cholesterol level was the same as the result found in the euthyroid females and this due to the fact that subclinical hyperthyroid subjects have normal level of T4 and T3 and hence normal function at the thyroid hormones. These finding agree with Caparevic et al., (2000). Also in this study there was no significance differences in all groups regarding the HDL-Cholesterol or LDL-Cholesterol level. These results to some extent are not surprising, since many studies, concerning patients with overt hyperthyroidism, reported that hyperthyroidism is associated with decreased or normal concentrations of total cholesterol and the other serum lipoproteins and lipids. Moreover, Hanson, (1983) studied the effect of experimental hyperthyroidism on plasma lipoproteins and stated that, the decreased LDL-cholesterol concentrations in hyperthyroid patients may be due to a mechanism include both an increased number of LDL-receptors and enhanced transfer of cholesterol and phospholipids to the LDL sub-fraction with subsequent rapid uptake in the liver via hepatic lipase. Another study carried by Caparevic et al., (2000) included 55 elderly patients with subclinical hyperthyroidism, concluded that patients with subclinical hyperthyroidism tend to have low serum total cholesterol, LDL-cholesterol and HDL-cholesterol levels. Also they found significant increase of serum cholesterol, LDL-cholesterol and HDL-cholesterol levels after treatment.

Relaying on these findings above, and the fact that thyroid hormones could increase lipolysis, it is fairly expected that the levels of serum lipids in patients with subclinical hyperthyroidism are decreased or at the normal range. In this study, subjects with subclinical hyperthyroidism, to some extent, are found to be having normal serum lipids, suggesting that there might not be relationship between subclinical hyperthyroidism and lipids in people whose close relatives are affected. Having other autoimmune disease, such as type I diabetes, vitiligo and Addison’s disease, increase the chance of getting hyperthyroidism (Kumar and Klark, 2002).
instituted without biochemical confirmation. Differentiation of the mild case from anxiety states may be difficult; useful positive clinical markers are: eye signs, a diffuse goiter, proximal myopathy and wasting, and hyper dynamic circulation with warm peripheries (Kumar and Clark, 2002).

**Biochemical investigations**

Serum TSH is suppressed in hyperthyroidism (< 0.05 mU/l) except for the very rare instances of TSH hyper-secretion. Diagnosis is confirmed with a raised serum T4, free T4 or T3. T4 is almost always raised but T3 is more sensitive as there are occasional cases of isolated T3 toxicosis. Thyroperoxidase (TPO) and thyroglobulin antibodies are present in most cases of Graves’ disease. TSH receptors antibodies are not measured routinely, but are commonly present. Thyroid stimulating immunoglobulin (TSI) in 80% is positive, TSH-binding inhibitory globulin (TBIG) 60-90% in Graves’ disease (Kumar and Clark, 2002).

**Table (1): Shows the symptoms of hyperthyroidism**

<table>
<thead>
<tr>
<th>Metabolic effects of thyroid hormones</th>
<th>Increased sympathetic activity</th>
<th>Unknown mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight loss</td>
<td>Nervousness</td>
<td>Goiter</td>
</tr>
<tr>
<td>Hair loss</td>
<td>Heat intolerance</td>
<td>Optimal nutrition</td>
</tr>
<tr>
<td>Thin skin</td>
<td>Sweating</td>
<td></td>
</tr>
<tr>
<td>Onycholysis</td>
<td>Tremor</td>
<td></td>
</tr>
</tbody>
</table>

This study was designed to compare the level of some serum lipid parameters (Cholesterol, TG, LDL-Cholesterol and HDL-Cholesterol) in hyperthyroid and subclinical hyperthyroid patients compared to euthyroid subjects. In the present study found that serum T3 with mean values 1,97, 2,19, and 7,4 nmol/L for euthyroid, subclinical and clinical hyperthyroid females respectively. While the mean values for T4 were found to be 94,30, 103,3, and 231,0nmol/L for euthyroid, subclinical and clinical hyperthyroid females respectively. These finding were in agreement with level reported by Series et al , (1988). In this study subject that considered as subclinical hyperthyroid cases have obviously suppressed TSH level. Diana and Kenneth , (2002) reported that the level of subclinical hyperthyroidism is due to the sensitivity of pituitary gland to respond to minor elevation in serum or tissue T4 and T3. Although the level of T3 and T4 remain within the normal range minimal
Chapter Four

Discussion

4.1 Survey

Almost 15% of the Sudanese females referred to RIA laboratory of SAEC during 1994 to 1996 were found to be under treatment which is the same results found in males bearing in mind that 87% of the referred patients were females and this is in a good agreement with what has been published (Suklar et al., 2000). 71.4% of the males were found to have euthyroid compared to 67.1% among the females who visited the laboratory in the same period that means females are more susceptible for thyroid disorders compared to the males. Those who had hyper active thyroid among the males were found to represent only 73 patients of 13.5% compared to 407 patients of 10.9% among the females. Subclinical hyperthyroid was found to be only 25 patients of 3.0% among the males and 93 patients of 2.3% among the females that is more or less the same percent of incidence. To have a comprehensive study with regard to the study of the prevalence of these disorders, a detailed register should contain many parameters in addition to the data used to collect from the referred patients that includes residence, tribe, family history for the disease or any endocrine disorder, treatment and any other informative data that can be used to reduce the risk factors and hence improve the quality of life for those who are under risk.

1.4 Subclinical hyperthyroidism

Subclinical hyperthyroidism is defined as persistently suppressed serum TSH with normal thyroxine and triiodothyronine in patients without典型的 symptoms (Kee et al., 2003).

While the diagnostic criteria and treatment modalities for overt hyperthyroidism are well known, the literature on assessment and treatment of patients with subclinical hyperthyroidism is markedly less extensive. The precise pathophysiology, natural history, risks and long-term outcome of subclinical hyperthyroidism are unknown (Diane and Kenneth, 2002).

The prevalence of subclinical hyperthyroidism varies amongst the reports. To date, there is no definitive information about the incidence of subclinical hyperthyroidism in the general population. Its prevalence ranges from 0.6 to 16% depending on diagnostic criteria, the sensitivity of the
1.4.1 Causes

Most patients with subclinical hyperthyroidism are ambulatory outpatients who are otherwise relatively healthy or have stable, chronic medical conditions. Abnormalities in the TSH remain for months or years in the absence of overt clinical symptoms (Diane and Kenneth, 2002).

The most common causes of subclinical hyperthyroidism are excessive thyroid hormone therapy. The causes can be endogenous as Graves' (early in its course) or it can be caused by thyroid conditions such as autonomous adenoma, multi-nodular goiter, or thyroiditis, or due to nonthyroidal conditions: euthyroid sick syndrome, acute psychiatric disease, pituitary and hypothalamic disorders, pregnancy, and drugs: thyroxine dopamine, glucocorticoids, aspirin and furosemide (Kek et al., 2003). In patients with toxic adenoma or multi-nodular goiter, subclinical hyperthyroidism is usually a slowly progressive disorder and may last several years before being diagnosed (Biondi et al., 2005).

1.4.2 Clinical implications

Increasingly, it is recognized that subclinical hyperthyroidism is not merely a biochemical abnormality dissociated from the clinical manifestations and squeal of thyrotoxicosis. In fact, clinical features of thyrotoxicosis can be identified in the subclinical hyperthyroidism (mg/dl) in the euthyroid and hyperthyroid Sudanese females. The clinical significance of subclinical hyperthyroidism thus relates to three risk factors: progression to overt hyperthyroidism, cardiac effects, and skeletal effects.
compounds that yield fatty acids on hydrolysis or complex alcohols that can combine with fatty acids to form esters. Some lipids contain non-lipids groups such as sialie, phosphpryl, amino or sulphate groups. The presence of these groups gives lipids molecules affinity for both water and organic solvents. This affinity is important in the formation of biological membranes (Ganong, 1997).

1.5.1 Classification of lipids

Lipids can be subdivided broadly into five groups:

1. Sterol derivatives: cholesterol and cholesterol esters, steroid hormones, bile acids, and vitamin D.

2. Fatty acids: short chain, medium chain, long chain, and prostaglandins.

3. Glycerol esters: tri-di- and mono-, glycerides; and phosphoglycerides.

the plasma lipoproteins and between cell membranes and lipoprotein, yet strong enough to allow the various classes and subclasses of lipoproteins to be isolated by a variety of analytical techniques.

**Classifications**

Lipoproteins have been categorized into five classes according to their physical and chemical properties and according to their chemical composition (%) as shown in table (2).

**Lipoprotein metabolism**

The pathways of lipoprotein metabolism are complex. The way these lipoproteins are organized into an exogenous pathway, which transport lipids from the intestine to the liver, and endogenous pathway, which transport lipid to and from the tissues.

1. **Exogenous pathway:** lipoproteins in this pathway of dietary origin. Chylomicrons are formed in the intestinal mucosa during the absorption of the products of fat digestion. They are very large lipoprotein complexes that enter the circulation via the lymphatic ducts. Chylomicrons are cleared from the circulation by the action of lipoprotein lipase which catalyzed the break down of triglyceride in the chylomicrons to fatty acids and glycerol, which then enter adipose cells and are reesterified. Chylomicrons depleted of their triglyceride remain in the circulation as cholesterol–rich lipoproteins called chylomicrons remnants, which are carried to the liver where they bind to LDL receptors and internalized by receptor mediated endocytosis and are degraded in lysosomes.

Figure (19): The prevalence of HDL-cholesterol (mg/d)/l in the euthyroid and hyperthyroid Sudanese females.

**Endogenous pathway:** This system made up of VLDL, IDL, LDL and HDL which transports triglycerides and cholesterol throughout the body. VLDLs are formed in the liver and transport triglycerides to extrahepatic
tissues. Triglycerides are removed by the action of lipoprotein lipase; they become IDL. The IDL give up phospholipids and through the action of the plasma enzyme lecithin cholesterol acyltransferase (LCAT) pick up cholesterol esters formed from HDL. The remaining IDL lose more triglyceride and protein and become LDL. LDL provide cholesterol to the tissue and taken up by receptor-mediated endocytosis (Tiez et al, 2001). LDLs are also taken up by a low-affinity system in the macrophages and some other cell (Ganong, 1997).

Table (2): The percentage of different complements for each lipoprotein

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Complement</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Apolipoproteins</th>
<th>Triglyceride</th>
<th>Cholesteryl esters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>90</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Figure (18): The mean values of HDL-Cholesterol (mg/dl) in the euthyroid and hyperthyroid Sudanese females.
1.5.1.2 Glycerol esters

Glycerol is a three-carbon alcohol with a hydroxyl group on each of its carbon atoms. The class of acylglycerol (glyceride) is determined by the number of fatty acyl groups present; one fatty acid (mono acylglycerol), two fatty acids (diglycerides) or three fatty acids (triglycerides). In human nutrition; triglycerides constitute 95% of tissue storage fat and are the predominant form of glyceryl ester found in plasma (Murray et al, 2000).

Metabolism

Triglycerides are digested in the duodenum and proximal ileum, through the action of pancreatic and intestinal lipases and in the presence of bile acid. They are hydrolyzed to glycerol, monoglycerides and fatty acids. After absorption, triglycerides are re-synthesized in the intestinal epithelial cells and combined with cholesterol and apo 3-48 to form chylomicrons, which are secreted to the lymphatic system, travel through the thoracic duct and eventually reach the blood stream through the jugular vein (Murray et al, 2000).

Familial hypertriglyceridemia

A moderate increase in the concentration of serum triglycerides is characteristic of familial hypertriglyceridemia (FHTG). The production of large VLDL-Cholesterol with abnormally high triglyceride content appears to be responsible for this disorder. The cholesterol content of VLDL-Cholesterol also increased but plasma LDL-Cholesterol and apo B-100 concentrations are within their reference intervals. This finding suggests that the conversion of VLDL-Cholesterol to LDL-Cholesterol is not increased in these individuals. Furthermore, plasma LDL-Cholesterol in those with FHTG often decreased dramatically, probably secondary to the hypertriglyceridemia. The cause of the
overproduction of VLDL-Cholesterol triglycerides is currently unknown. The diagnosis of familial hypertriglyceridemia (FHTG) requires study of other family members to differentiate this disorder from FCHL. This disorder appears to be inherited in an autonomic dominant pattern.

1.5.2 Effects of hyperthyroidism on lipids metabolism

Alterations of the lipid profile are well known phenomena in thyroid dysfunction. Thyroid hormones regulate lipid metabolism through various mechanisms. Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels (Spandrio et al., 1993).

It has been known for over fifty years that an increase in thyroid
individuals who have a cholesterol level greater than 34 mIU/ml and low thyroxine 45 nmol/l this data suggesting that thyroid dysfunction may make a significant contribution to hypercholesterolemia in general population (Series and Biggart, 1988). A study carried out by Duntas and Leonidas, (2002) showed that hyperthyroidism exhibits an enhanced excretion of cholesterol and increased turnover of LDL resulting in a decreased total and LDL-cholesterol, whereas HDL is decreased or not affected. Also, the composition and the transport of lipoproteins are seriously disturbed in thyroid diseases. Rassoul, et al., (1988) postulated that clinical manifestation of hypothyroidism lead to changes of plasma lipoproteins, which are characterized by elevated LDL-cholesterol, an increase of the ratio of LDL-cholesterol/ HDL-cholesterol. On the contrary patients with hyperthyroidism showed low lipoproteins levels (Rassoul et al., 1988). Also Duntas and Leonidas, (2002) reported that, there is a marked increase in HDL levels in hyperthyroid patients due to increased activity of (CETP) and hepatic lipase (HL) which are regulated by thyroid hormones. They suggested that the low activity of (CETP) and more specifically of (HL), results in reduced transport of cholesteryl esters from HDL to VLDL and IDL and reduces transport of HDL2 to HDL3 (Duntas and Leonidas, 2002).

Figure (15): The prevalence of TG (mg/dl) in the euthyroid and hyperthyroid Sudanese females.
CHAPTER TWO

Materials and Methods

2.1 The Survey

A general survey was carried out for the records of thyroid function test to subjects visited the SAEC laboratories during 1994-1996. The study focused on the variation in sex, age and subject's status, whether they were new cases or under treatment this is related to their thyroid status. The statistical analysis was done using statistical package for social science (spss) and excels software of Microsoft.

2.2 The effect of clinical and subclinical hyperthyroidism on lipids profile

2.2.1 Subjects

One hundred and twenty adult female patients their ages ranging between Figure (14): The mean values of TG (mg/dl) in the euthyroid and hyperthyroid Sudanese females.
and stored at -20 °C for the determination of triglycerides and total cholesterol and its fractions.

2.3 Reagents

The levels of TSH, T4, and T3 were estimated by using Radioimmunoassay (RIA) techniques, (RIA) kits are all supplied by the Department of Isotopes, China Institute of Atomic Energy, 1994. Each Kit has specific code: IMK-437, IMK-438, and IMK-432 for T4, T3, and TSH respectively.

2.3.1 T4 kits

Each kit consisted of 6 vials of lyophilized standards of T4 to give different concentrations when reconstituted by 1 ml of distilled water; (0, 26, 52, 103, 206, and 309 nmole/l thyroxin). One vial of T4-I\(^{125}\) tracer and T4 antibodies coupled to magnetic particles, and one vial for quality control sample (QC).

2.3.2 T3 kit

Each one includes 6 vials of triiodothyronine standard solution in different concentrations, (0, 0.5, 1.0, 2.0, 4.0, 8.0 nmol/l triiodothyronine). One vial contains T3-I\(^{125}\) tracer, one vial Anti-T3 antibody, and one vial of QC sample.

2.3.3 Thyroid stimulating hormone (TSH) kit

Each kit contains 7 vials of standard solution of TSH which has the following concentrations: 0.0, 0.23, 1.0, 3.0, 10.0, 20.0 and 80.0 mIU/ml. One vial contains anti-TSH antibodies that has been coupled to magnetic particles; solid phase separation system. One vial of tracer labeled anti-TSH with I\(^{125}\); one vial wash Buffer, 3 vials of QC samples A, B and C with different ranges.

3.2.6 Low density lipoproteins cholesterol

LDL-cholesterol showed different results from HDL-cholesterol; euthyroid and subclinical hyperthyroid were found to have almost the same results where majority of the samples were found to have normal levels of LDL-cholesterol with \((p>0.05)\) which means that no significant variation while 42.5% of the hyperthyroid females were found to have low levels of LDL-cholesterol and 55% of them found to have normal levels \((p<0.001)\) which means that significant variation occurred between the euthyroid and subclinical hyperthyroid females. The mean values were found to be 104, 95 and 75 mg/dl for euthyroid, subclinical hyperthyroid and clinical hyperthyroid
hyperthyroid females; results are almost the same as the results found in the euthyroid females and this is due to the fact that subclinical hyperthyroid subjects have normal levels of T4 and T3 and hence normal functions of the thyroid hormones, p value was found to be (p>0.05) which means that there is no significant variation between the two groups. The mean values of TC levels were found to be 172.7 in the euthyroid females which is the same finding in the subclinical hyperthyroid females compared to 141.7 mg/dl in case of hyperthyroid females. It is obvious that TC levels are affected by the clinical hyperthyroid function, which lead to a decrease in its levels in the majority of samples collected from hyperthyroid females; also the mean value was found to be less than the lower limit of the normal range. However, subclinical hyperthyroid results showed the same results that found in the euthyroid females, both of them have mean values that within the normal range. The mean values were shown in figures 16 and table 5 while the prevalence in the different groups was shown in figure 17. The lowest level of TC was reported in the hyperthyroid patients, which was lower than the normal range whereas the subclinical cases kept the same value as in the euthyroid subjects.

3.2.5 High density lipoprotein cholesterol

In case of HDL-cholesterol, significant variation was observed between the euthyroid and hyperthyroid females with (p>0.05) and the mean values found to be 52.1 and 44.8 mg/dl for euthyroid and clinical hyperthyroid females respectively but no significant variation was found between the euthyroid and subclinical hyperthyroid females (p>0.05) and mean value equal to 48.60 mg/dl). These findings showed that thyroid dysfunctions affect high density lipoprotein cholesterol among the hyperthyroid females but not the euthyroid ones.

2.3.4 Reagents for lipids’ measurement

Commercial enzymatic methods were used for determination of total cholesterol, triglycerides, LDL-Cholesterol and HDL-Cholesterol. All kits and reagents were supplied by (Biosystems) company, for reagents and instruments. Methods and procedures are applied according to the instructions described in the kits manuals. Each Kit has specific code: 11570, 11648, 11579, and 11528 for Cholesterol, HDL-Cholesterol, LDL-Cholesterol and TG respectively.

2.4 Equipments:

1. Adjustable micropipettes (10-200µl) with disposable tips.
2. Polystyrene test tubes (disposable).
3. Vortex mixer (single and multi-tubes).
5. Water bath.
After mixing well, the tubes were incubated at 37 °C for 45 minutes, then the rack was placed on the magnetic base for 10 minutes to separate the bound fraction from the free fractions by decant the supernatant. Lastly each tube was counted in the gamma counter to evaluate the gamma emission per minute, and binding percent was plotted vs. the concentration of the corresponding standard to get a standard calibration curve, and from this curve, thyroxin concentration of the unknown samples can be obtained. This method is bioassay method, (Radioimmunoassay), Using radio active isotope of iodine (I\(^{125}\)) which is gamma emitter (Edward, 1980).

2.5.2 T\(_3\)

Test tubes were labelled in duplicate and arranged in assay rack. 25 µl of standard solutions, QC samples and patient samples were added to the corresponding tube. 250 µl of T\(_3\)-I\(^{125}\) tracer and 100 µl anti-T\(_3\) antibodies were added to each tube and mixed. The tubes were incubated at 37 °C for one hour and then well vortexed and then centrifuged to separate bound fraction; liquid phase separation system, the supernatant was decanted and then each tube was placed in gamma counter. The principle of the assay is the same as that for T\(_4\) (Edward, 1980).

2.5.3 TSH

Test tubes were labeled and arranged in assay rack in duplicate. 100 µl of STD, QC and samples were pipetted in target tubes, 25 µl tracer (anti-TSH labeled by I\(^{125}\)) were added to each tube and vortexed gently, and then incubated at 37 °C in the incubator for one hour. 250 µl of anti TSH (antibody coupled to magnetic particles) was added to each tube and mixed well and incubated at 25 °C for one hour. Then the racks were placed in the magnetic separator for 10 minutes and the supernatant was separated by decantation.

3.2.3 Results of triglycerides (TG)

When results of triglycerides were analyzed, 37.5% of the euthyroid females were found to have low levels that were less than the lower limit of the normal range compared to 57.5% that have normal levels of TG and only 5% that have high levels of TG. In case of subclinical hyperthyroid females, 82.5% were found to have normal levels of TG while 37.5% were found to have high levels of TG and none of them was found to have low TG. The same results were found in the hyperthyroid females. The mean values of TG levels were found to be 92.6, 152.9 and 146.1 mg/dl in euthyroid, subclinical hyperthyroid and clinical hyperthyroid females respectively. Almost all the mean values were found to be within the normal range of TG but statistical analysis showed that there is highly significant variation between euthyroid and hyperthyroid results (p < 0.001) and the same result was found between euthyroid and subclinical hyperthyroid results. TG mean values were shown
Figure (13): The prevalence of TSH mlu/ml levels among the euthyroid and hyperthyroid Sudanese females

Washing step

In this step first the concentrated wash buffer was diluted by adding water (1:9), and then 500 µl of the diluted wash buffer was added to each tube, vortexed well and then placed again in the magnetic base and allowed to stand for 10 minutes. The supernatant was decanted and drained thoroughly on adsorbent paper. The wash step was repeated again. All the tubes were counted in the gamma counter, to evaluate the concentration of TSH in the patient sample.

The quantitative analysis of TSH is achieved by the above method, which is immunoradiometric method. It is non-competitive method in which the radio active compound (tracer) is TSH antibody. There are two antibodies to react with the TSH in the analyte to get a sandwich complex (Edward, 1980).

2.5.4 Calculation of results
2.6.1 Estimation of Total Cholesterol

**Principle of the method**

In the presence of Cholesterol esterase, the cholesterol esters in the sample are hydrolyzed to cholesterol and free fatty acids. The cholesterol produced is oxidized by Cholesterol oxidase to cholestenone and hydrogen peroxide. Hydrogen peroxide is detected by a chromogenic oxygen acceptor, phenol- ampyrone, in the presence of peroxidase. The red quinine formed is proportional to the amount of cholesterol present in the sample (Tietz et al, 2000).

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acid}
\]

\[
\text{Cholesterol} + \frac{1}{2} \text{O}_2 + \text{H}_2 \xrightarrow{\text{CHOD}} \text{Cholesterol} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoniemine} + 4\text{H}_2\text{O}
\]

**Contents and composition of reagents**

1. **Reagent-1 (Buffer):** PIPES PH 6.9 90 mmol/l, phenol 26 mmol/l

2. **Reagent-2 (Enzymes):** Cholesterol esterase (CHE) 300 U/ml, Cholesterol oxidase (CHOD) 300 U/L, Peroxidase (POD) 1250 U/ml, 4-aminoantipyrine (4-AP) 0.4 mmol/l, pH 7.0.

3. **Cholesterol standard (200 mg/dl).**

**Procedure:**

![Figure](image-url)
Figure (11): The prevalence of T₃ (nmol/l) levels among the euthyroid hyperthyroid Sudanese females

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard (ST)</th>
<th>Sample (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol standard</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthyroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subclinical Hypothyroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sample</strong></td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
<tr>
<td><strong>Working reagent</strong></td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

2.3- The tubes were incubated for 10 minutes at room temperature (25°C).

3.4- The absorbance (A) of the standard and samples were measured at 500 nm against the blank.

**Calculations:**

\[
\frac{A_s \times 200}{A_{std}} = \text{cholesterol (mg/ dl)}
\]
Procedure

Precipitation

1. Into labeled centrifuge tubes the following were pipetted:

<table>
<thead>
<tr>
<th>Sample</th>
<th>0.2 ml</th>
<th>Reagent B</th>
<th>0.5 ml</th>
</tr>
</thead>
</table>

2. The tubes were left to stand for 10 minutes at room temperature.
3. Centrifuged at minimum of 4000 r.p.m for 10 minutes.
4. The supernatant was collected carefully.

Colorimetry

1. The cholesterol reagent was brought to room temperature.
2. Into labeled test tubes the following were pipetted:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard (ST)</th>
<th>Sample (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>100 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HDL-Cholesterol standard</td>
<td>-</td>
<td>100 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample supernatant</td>
<td>-</td>
<td>-</td>
<td>100 µl</td>
</tr>
<tr>
<td>Cholesterol reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
</tbody>
</table>

3. The tubes were incubated for 10 minutes at 37°C.
4. The absorbance (A) of the sample and the standard were measured at 500 nm against the blank.

Calculations:

As X 3.5 X 40
A std = HDL-Cholesterol (mg/dl)
Figure (9): The prevalence $T_4$ (nmol/l) level among the euthyroid and hyperthyroid Sudanese females

![Bar graph showing prevalence of $T_4$ levels among different thyroid statuses.]

2.6.3 Estimation of Low-density Lipoprotein Cholesterol

**Principle of the method:**

Low-density lipoproteins in the sample are precipitated with polyvinyl sulphate. Their concentrations are calculated from the difference between the serum total cholesterol in the supernatant after centrifugation. The cholesterol is spectrophotometrically measured by means of the coupled reactions as described in total cholesterol (Tietz, 1999).

**Contents and composition of reagents**

1. Reagent B: 10 ml polyvinyl sulphate 3 g/L, polyethylene glycol 3 g/L.
2. Cholesterol kit (Biosystem code 11505, 11506, 11539).

**Procedure**

**Precipitation**

1. Into labeled centrifuge tubes the following were pipetted:
3- The tubes were mixed and incubated at room temperature for 30 minutes.

4- The absorbance (A) of the sample and the standard were measured at 500 nm against the blank.

**Calculations**

The dilution factor of the sample in the precipitate is 1.5 and the concentration of the standard is 200 mg/dL according to the following formula is;

\[ \text{Cholesterol (mg/dl)} = \frac{\text{AS} \times 200}{\text{AST}} \]

\[ \text{LDL-Cholesterol} = \text{Total-cholesterol} - \text{supernatant cholesterol}. \]

**2.6.4 Estimation of Triglycerides**

**Principle of the method**

Triglycerides in the sample originate, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometer (Fossati and Precipe, 1982).

\[ \text{Triglycerides} \rightarrow \text{H}_{2}\text{O} \rightarrow \text{lipase} \rightarrow \text{Glycerol + Fatty acids} \]

\[ \text{Glycerol} + 3\text{ATP} \rightarrow \text{glycerol kinase} \rightarrow \text{Glycerol-3-p + 3ADP} \]

\[ \text{Glycerol-3-p + O}_2 \rightarrow \text{G3p-oxidase} \rightarrow \text{Dihydroxyacetone-p + H}_{2}\text{O}_2 \]

\[ \text{2H}_2\text{O}_2 + 4\text{-Aminoantipyrine + 4-cholesterol} \rightarrow \text{peroxidase Quinoneimine + 4H}_2\text{O} \]

**Figure (8): The mean values of T4 in the euthyroid and hyperthyroid Sudanese females**

<table>
<thead>
<tr>
<th>Mean T4 (nmol/l)</th>
<th>Euthyroid</th>
<th>Subclinical Hyperthyroid</th>
<th>Clinical Hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(11) show the same results for the $T_3$, and the TSH results were shown in figures (12) and (13).

Table (4): shows the levels of thyroid and thyroid related hormones in euthyroid and hyperthyroid Sudanese females Expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Thyroid status</th>
<th>Thyroxine</th>
<th>$T_3$</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal ranges</strong></td>
<td>50-150 nmole/L</td>
<td>0.80-3.0 nmole/L</td>
<td>0.40-4.0 mIU/ml</td>
</tr>
<tr>
<td><strong>Euthyroid</strong></td>
<td>94.30 ± 9.97</td>
<td>1.97 ± 0.46</td>
<td>1.60 ± 0.54</td>
</tr>
<tr>
<td><strong>Hyperthyroid</strong></td>
<td>231.80 ± 48.49</td>
<td>7.40 ± 2.87</td>
<td>0.18 ± 0.50</td>
</tr>
<tr>
<td><strong>Subclinical hyperthyroid</strong></td>
<td>103.30 ± 15.23</td>
<td>2.19 ± 0.50</td>
<td>0.18 ± 0.08</td>
</tr>
</tbody>
</table>

- Same letters mean non significant
- Different letters mean significant across the columns

glycerol-3-phosphate oxidase > 4 U/ml, peroxidase > 0.8 U/ml, 4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.5.

2. Triglycerides standard. Glycerol equivalent to 200 mg/dl triolein.

**Procedure**

1- The reagent was brought to room temperature.

2- Into labeled test tubes the following were pipetted:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard (ST)</th>
<th>Sample (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triglycerides standard</strong></td>
<td>-</td>
<td>10µL</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sample</strong></td>
<td>-</td>
<td>-</td>
<td>10µL</td>
</tr>
<tr>
<td><strong>Reagent</strong></td>
<td>1.0mL</td>
<td>1.0mL</td>
<td>1.0mL</td>
</tr>
</tbody>
</table>

3- The tubes were incubated for 15 minutes at room temperature.

4- The absorbance (A) of the standard and sample was measured at 500 nm.


CHAPTER TREE

Results

3.1 The survey

3.1.1 Statistical analysis of the patients who referred to RIA laboratory, of SAEC (1994-1996)

From 1994 to 1996, 4268 Sudanese patients suspected to have thyroid disorders were referred to the RIA laboratory of SAEC for thyroid function test; among them 3730 females representing 87.4% compared to 538 males represent 12.6%. The average age was found to be 31 years as shown in figure (2). Almost 85% of the referred patients visited the RIA laboratory for the first time as shown in table 3. Figure (1) shows the type of visit to the RIA laboratory of SAEC during the period 1994 to 1996. However, figure (3) shows the prevalence of clinical and subclinical hyperthyroid compared to the euthyroid for both males and females. When analyzing the results by sex, males were found to represent 12.6% of the total patients referred to the lab.

3.2.2 Thyroid function test status in the study group of Sudanese females

A total of one hundred and twenty females were selected to participate in the present study 40 hyperthyroid females, equal number fit the criteria for subclinical hyperthyroidism cases, and the remaining 40 were euthyroid selected as controls.

Mean values of T4 were found to be equal to 94.30, 103.3 and 231.0 nmole/L for euthyroid, subclinical hyperthyroid and clinical hyperthyroid females respectively, significant variation was found between normal and hyperthyroid females for T4 but no significant variation between the normal and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively. T3 results showed 100% agreement in the case of euthyroid and subclinical hyperthyroid females for T4 (p<0.001) and (p>0.05) respectively.
Figure (7): The percent of each age group for the females who participated in the study.

Table (3): The percentage of males to females of Sudanese subjects who visited RIA Lab, SAEC during (1994-1996).

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Under treatment</th>
<th>New cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Male</td>
<td>80</td>
<td>14.87</td>
<td>458</td>
</tr>
<tr>
<td>Female</td>
<td>544</td>
<td>14.58</td>
<td>3186</td>
</tr>
<tr>
<td>Total</td>
<td>624</td>
<td>14.71</td>
<td>3644</td>
</tr>
</tbody>
</table>
Figure (1): The percentage for each type of visit to the RIA laboratory for the subjects under survey

3.2 Effect of clinical and subclinical hyperthyroid on lipid in Sudanese females

3.2.1 Age of females under study

The age of Sudanese females under study were analyzed statistically into three groups where 32.5% (39) of them were found to be less than 30 years old while 34.2% of them have ages ranged between 30 and 39 and 33.3% of them their ages ranged between 40 and 49 years as shown in figure(7).
Figure (6): The mean level of TSH (mIU/ml) of euthyroid and hyperthyroid subjects under survey

Figure (2): The mean of age for subjects under survey
Figure (3): The prevalence of hyperthyroid (clinical and subclinical) compared to euthyroid among Sudanese population

Figure (5): The mean of T₃ (nmole/L) of euthyroid and hyperthyroid subjects under survey
Figure (4): The mean of T₄ in (nmole/L) of euthyroid and hyperthyroid subjects under survey

Figure (4), (5) and (6) show the mean values of T₄, T3 and TSH for subjects under survey classified as euthyroid, clinical hyperthyroid and subclinical hyperthyroid; the results showed good agreement with the definitions of each class.